

**[0093]** The results showed substantially improved sensitivity using the ProteoMiner™ method of the present application. Although most of the proteins measured were present at very low level before ProteoMiner™ treatment, the relative concentrations of the same proteins were substantially increased after the ProteoMiner™ treatment. The sensitivities in detecting these low abundance proteins were improved significantly, since most HCPs were enriched more than 100 fold as shown in Tables 10 and 11. In particular, one selected HCP was enriched more than 1000 fold as shown in Table 10. The ProteoMiner™ method of the present application was able to increase the signal of each HCP significantly to reduce the dynamic range of the protein concentrations in the sample.

Example 7. Comparison Between Filtration Method and ProteoMiner™ Method

**[0094]** The efficiencies of HCP enrichments were compared between ProteoMiner™ and filtration (filter) method

for the detection and identification of HCPs in samples containing antibodies. 1.5 mg of mAb3 and HCPs were dissociated in SDC and SLS cocktail buffer, after dissociation, the HCPs can be separated from antibody by applying 50K MWCO filter. (Chen et al. Improved Host Cell Protein Analysis in Monoclonal Antibody Products through Molecular Weight Cutoff Enrichment. *Analytical Chemistry* 2020 92 (5), 3751-3757). The numbers of HCPs which were identified with two peptides are shown in FIG. 5A and FIG. 5B for filter and ProteoMiner™ methods (The IP result is the combined result that from all different lots of mAb3 and mAb4.). The testing results of the spiked-in HCPs were shown in Table 12. Thirteen purified HCPs from CHO cells with varied concentrations ranging from 0.1 ppm to 200 ppm were spiked into samples containing purified mAb3 for testing. As shown in Table 12, ProteoMiner™ method showed higher identified PSM and higher unique peptides overall.

TABLE 12

Comparison between Filter method and ProteoMiner™ method for spiked-in HCPs						
Spiked-in Final ppm	Protein Name	Molecular Weight	Identified psm (filter)	Unique Peptides (filter)	Identified psm (ProteoMiner™)	Unique Peptides (ProteoMiner™)
200	Beta-hexosaminidase	60.1k	64	10	116	22
100	Carboxypeptidase	54.2k	240	25	268	30
50	hPLBD2	65k	8	3	362	11
20	Cathepsin Z	34k	62	17	327	16
10	SIAE	61.4k	n/a	n/a	81	15
10	Cathepsin D	44.1k	12	4	211	16
5	Metalloproteinase inhibitor 1	22.4k	13	3	46	8
5	LAL (half dimer/monomer)	45.6k	n/a	n/a	99	10
5	peptidyl-prolyl cis-trans isomerase	23.6k	96	11	108	12
1	c-x-c motif chemokine	11k	31	4	26	3
1	Transthyretin	17k	34	4	36	6
0.5	Acid ceramidase	44.7k	n/a	n/a	52	12
0.1	Procollagen C endopeptidase enhancer 1	55.2k	n/a	n/a	4	2

Example 8. Repeatability Test

**[0095]** The repeatability of the ProteoMiner™ method of the present application for HCP enrichment was tested using mAb4. 15 mg of samples containing mAb4 and HCP impurities were tested using 1/5 of the ProteoMiner™ kit. Three replicates were conducted. As shown in Table 13 and FIG. 6, the results show good repeatability based on match to IP results.

TABLE 13

Repeatability tests of ProteoMinerTm method for HCP enrichment						
			High Confidence (>2 pep)	DS PSM (heavy/light)	Match to IP result (>2 pep) (total 48 targets from IP)	Match to IP result (include 1 pep) (total 48 targets from IP)
Sample Amount	Peptide Amount	Total ID				
1. 15 mg	21.7	142	73/(12)	9733/4152	19	24
2. 15 mg	24.5	137	62/(10)	10580/4579	19	25
3. 15 mg	22.35	138	74/(9)	11264/4845	22	24